Proteolytische activiteit bij neutrale pH in rundermilt

Marrink, Jan

SUMMARY

Intracellular enzymes, hydrolysing proteins optimally at acid pH values (acid proteases), have been studied in detail by several investigators. The existence of proteolytic activity at neutral pH in animal tissue extracts, on the other hand, has often been mentioned, but with few reports on the isolation, separation or characterization of the enzymes concerned. This is partly due to the fact that, the activities at acid pH values are higher than those at neutral pH, and also, due to the rather elusive nature of the enzyme(s) assumed to be active at neutral pH. Our studies were aimed at (1) the demonstration of the proteolytic activity, possibly present in beef spleen at neutral pH, and more especially, (2) the possible existence of an endopeptidase, active at neutral or slightly alkaline pH values.

In Chapter I, (a) the classification of proteolytic enzymes; (b) a general survey of the relevant literature on proteolytic enzymes in animal system; and, (c) a detailed survey of the intracellular neutral proteases, are given.

The materials and methods used, are described in detail, in Chapter II.

In Chapter III, our preliminary results concerning proteolytic activity at neutral pH in bovine spleen extracts, are given. It was shown, that, the observed increase in the absorbance at 280 nm – using the original method of Kunitz with casein as a substrate – was not due to proteolytic activity, but due to the following phenomena:

1° the formation of nucleotides from spleen RNA by ribonuclease present in casein preparations,

2° the action of endogenous ribonuclease on endogenous RNA,

3° the oxidation of certain components in the reaction mixture.

These effects, contributing to the absorbance at 280 nm, simulated the existence of proteolytic activity at neutral pH, thus leading to erroneous conclusions. The lack of reaction, of the products formed, with the Folin-Ciocalteu reagent (showing that aromatic amino acids are not released), was a final proof of the unsuitability of the E_{280}-method.

The surprising finding, of the presence of ribonuclease in commercial preparations of casein, is described in detail in Chapter IV. Most casein samples were found to contain about 20 μg ribonuclease per gram, and none was free of this contaminating enzyme. The interfering activity of ribonuclease can be eliminated by heating the casein solution for 15 min at 100° at pH 12.

By using a more specific method, viz. the reaction with the ninhydrin reagent on amino groups, we were able to demonstrate proteolytic activity at

neutral pH in spleen extracts (Chapter V). The highest yield of this activity in soluble form was obtained by homogenization at pH 5, followed by extraction at pH 8 of the pH 5 sediment. The active extract, still containing the endogenous substrate, has a pH optimum of 7.6 at 55° (optimum temperature) and 38°. The reaction products appeared to be mainly amino acids, but the presence of minor amounts of peptides could not be excluded at that stage.

A clear-cut separation of enzyme and substrate could be achieved by exhaustive digestion of the substrate at 45° in a dialysis tube. This enzyme preparation is active only when substrates are added. The original activity can be nearly completely restored by adding boiled extract which apparently contains a saturating amount of endogenous substrate.

Amino acid amides, peptides and some proteins were used as substrates. The results show clearly that spleen contains an unspecific *aminopeptidase* capable of splitting proteins into amino acids. On the basis of competition experiments with different substrates we can ascribe all activities observed to a single enzyme or a group of enzymes with the same qualitative specificity. The enzyme – which is not influenced by Mg^{++}, Mn^{++} and EDTA – has many features in common with known aminopeptidases and resembles in its specificity the classical pig kidney leucine aminopeptidase.

Our enzyme can account for all proteolytic activity at neutral pH observed in beef spleen extracts. The assumption of endopeptidases active at neutral pH, for which we obtained no evidence whatsoever, thus becomes unnecessary. The results of other investigators concerning proteolytic activity at neutral pH in tissue extracts are discussed in the light of our results (Chapter VI). We conclude that there is no compelling evidence for the existence of neutral *endo*peptidases in animal tissues.